PHOSPHOINOSITIDE 3-KINASES

BACKGROUND OF THE INVENTION

Phosphoinositide 3-kinases (PI3Ks) are ubiquitous lipid kinases playing key roles both as signal transducers downstream of cell-surface receptors and in constitutive intracellular membrane and protein trafficking pathways. All PI3Ks are dual specificity enzymes with a lipid kinase activity capable of phosphorylating phosphoinositides at the 3-hydroxyl and with a protein kinase activity. The products of PI3K-catalysed reactions, phosphatidylinositol 3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$), $PtdIns(3,4)P_2$ and PtdIns(3)P act as second messengers for a variety of signal transduction pathways, including those essential to cell proliferation, adhesion, survival, cytoskeletal rearrangement and vesicle trafficking (1,2).

The mammalian PI3Ks can be divided into three classes based on their structure and substrate specificity (2). The class I PI3Ks are receptor-regulated heterodimeric enzymes that preferentially phosphorylate $PtdIns(4,5)P_2$ *in vivo*. The class IA PI3Ks (consisting of p110α, p110β, or p110δ catalytic subunits) associate with an 85 kDa adaptor that is essential for interaction of these PI3Ks with receptor tyrosine kinases. The class IB PI3K (PI3Kγ) is activated by heterotrimeric G protein subunits and associates with a p101 adaptor that is important for full responsiveness to Gβγ heterodimers (3,4). Class I PI3Ks are also activated by Ras. Class II PI3Ks are distinguished by a C-terminal C2 domain and preferentially use PtdIns and PtdIns(4)P as substrates. Class III enzymes phosphorylate only PtdIns and lack the Ras-binding domain.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows the overall structure of PI3KC (A) A ribbon diagram of PI3K (prepared with MOLSCRIPT) showing the four domains: the RBD, the C2 domain, the helical domain and the catalytic domain with N-lobe and C-lobe. The N-terminal region preceding the RBD and the ordered portion between the RBD and C2 domain are white. (B) The solvent-accessible surface of the enzyme in the same orientation (prepared with GRASP (29)). (C) A block diagram showing the domain organization of the PI3K classes.

Fig. 2 shows a schematic representation of the catalytic domain of PI3K. (A) A ribbon diagram of the PI3K catalytic domain with bound ATP. The two disordered residues in the middle of the activation loop are represented by dotted lines. (B) The active conformation of the Src family protein kinase Lck (30) (PDB entry 3lck). (C) A stereo representation of PI3K active site with bound ATP and two Lu^{3+} ions (labelled Me I and Me II).

Fig. 3 show the complete amino acid sequence of a porcine PI3Kγ. The (.....) indicate gaps when the PI3Kγ is aligned with other members of the PI3K family. The other PI3Ks are not shown, but are incorporated by reference to Walker et al., Nature, 402:313-320, 2000.

Fig 4 is a model of phospholipid headgroup interactions with PI3K. (A) Two views of the solvent-accessible surface of the enzyme. The activation loop is coloured black. An inositol 1,4,5-trisphosphate ($InsP_3$) molecule (white ball-and-stick) has been modelled in the active site with the 3-OH near the γ-phosphate of the bound ATP. (B) The same two views of the enzyme in ribbon representation with the activation loop and $InsP_3$ cyan. The right portion of the panel has been expanded to illustrate some features of the putative headgroup interaction.

Fig. 5 shows a model of the Ras/PI3K interaction based on the structure of the RalGDS/Ras complex. The inset shows an overall view of Ras/PI3K interaction. Residues in Switch I and Switch II regions of Ras that influence effector binding are highlighted with stripes, while residues in the RBD of PI3K that are likely to be involved in Ras binding are shown as black stripes. The proximity of the RBD to the two lobes of the catalytic domain is also illustrated.

Fig. 6 is a ribbon diagram of the PI3KγC2 domain and the interactions it makes with the remainder of the enzyme. The elements of the helical and catalytic domains interacting with the C2 domain are shown. The inset shows the area selected for the detailed illustration.

Fig. 7 is a schematic illustration of the helical domain. The A/B anti-parallel helical pairs characteristic of the HEAT motif topology consist of hA1/hB1, hA2/hB2, hA3/hB3, hA4/hB4 and hA5/hB5. The left half of the panel illustrates the interaction that the helical domain makes with the RBD and C2 domain (the remainder of the protein was removed for clarity). This interaction involves principally the A-helix

surfaces. The interactions between the helical domain and the catalytic domain are shown on the right.

Fig. 8 shows the complete amino acid sequence of porcine PI3Kγ.

DESCRIPTION OF THE INVENTION

The present invention relates to phosphoinositide 3-kinases (PI3Ks), a class of enzymes involved in signal transduction and in constitutive intracellular membrane protein trafficking pathways. PI3Ks possess dual catalytic functions, possessing both lipid kinase and protein kinase activity. The products of PI3K-catalyzed reactions are second messengers in a variety of signal transduction pathways, including those involved in cell proliferation, adhesion, survival, cytoskeletal rearrangement, and vesicle trafficking. Thus, modulating its activities is useful for regulating cellular activities, e.g., involved in inflammation, repair, healing, development, and differentiation (e.g., for regulating stem cell growth and differentiation).

In accordance with the present invention, the three dimensional structure of a PI3Kγ has been determined. The present invention thus relates to a PI3Kγ crystal with unit dimensions of about a=143.3 Å, b=67.6 Å, c=107.0 Å, and β=95.9°. The crystals have C2 symmetry and contain one molecule in the unit cell. Crystals can be grown and analyzed by any effective methods, such as methods described in the examples below.

The present invention also relates to PI3Kγ polypeptide muteins, polypeptide fragments, antibodies thereto, nucleic acids coding for these polypeptides, methods of modifying PI3Kγ activity, and methods of modulating PI3Kγ activity. These include polypeptides and methods thereof, relating to, e.g., phospholipid binding, lipid kinase activity, modulating Ras activity in activating the PI3Kγ, binding of PI3Kγ to cell membranes, and modulating protein-protein interactions with PI3Kγ. Polypeptides, nucleic acids, and antibodies can be prepared according to any effective method.

By the term "mutein," it is meant any non-naturally occurring mutation. Mutations can be introduced by any suitable method, e.g. by site-directed mutagenesis, by routinely by modifying or mutating a nucleotide sequence coding for an amino acid sequence of Fig. 3, and selecting for those mutations that affect one or more of its activities, e.g., by measuring activity as described in Bondeva et al.,